

## First Record of Three *Loxodes* Ciliates (Ciliophora: Loxodida: Loxodidae) from Korea

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### ABSTRACT

Three *Loxodes* ciliates collected from estuarine littoral, wetland and small pond in Korea, were identified as *Loxodes kahli* Dragesco and Njiné, 1971, *L. magnus* Stokes, 1887 and *L. vorax* Stokes, 1885. The descriptions for these species based on living and protargol impregnated specimens were given. Morphometry, illustrations and microphotographs were also provided. Diagnoses of three species are as follows. *Loxodes kahli*: size *in vivo* 160-300 × 40-70 µm; oral area with reddish to brownish pigments; 6-11 macronuclei arranged linearly; 5-9 micronuclei located near macronuclei; 4-12 Müller's vesicles; somatic kineties on right 18-20 and left 2 in number. *L. magnus*: size *in vivo* 250-470 × 87-15 µm; body colored dark brown; 5-13 macronuclei; 8-13 micronuclei; 8-18 Müller's vesicles; somatic kineties on right 23-26 and left 2 in number. *L. vorax*: size *in vivo* 70-160 × 20-35 µm; oral area with brownish pigments; 2 macronuclei; 1 micronucleus located between macronuclei; 2-4 Müller's vesicles; somatic kineties on right 18-20 and left 2 in number.

**Key words:** *Loxodes*, morphology, redescription, Korea

### INTRODUCTION

The genus *Loxodes* is assigned to the family Loxodidae, order Loxodida and class Karyorelictea within phylum Ciliophora. The family Loxodidae is characterized by a long and laterally flattened shape with a beak-like anterior rostrum which interrupts perioral kineties at the anterior end and Müller's vesicles in the cytoplasm. Within the family Loxodidae, two genera *Loxodes* and *Remanella* have been recognized until now. The genus *Loxodes* is differentially diagnosed by the round shape of an oral area behind a rostrum which leads into a tubular cavity lined by extensions of oral dikinetids, inhabiting freshwaters, while the genus *Remanella* characterized by a long oral area which leads into a tubular cavity not lined by extensions of oral dikinetids, inhabiting brackish and marine waters (Curds, 1982; Small and Lynn, 1985; Carey, 1992; Lynn, 2008). Since the establishment of the genus *Loxodes* by Ehrenberg (1830), nine species have been described worldwide but there is no full revision on this genus. In this study, three *Loxodes* species, *L. kahli*, *L. magnus* and *L. vorax*, were isolated and reported for the first time in Korea. Although these three species are common in aquatic environments, they have never been described in Korea. For the researchers trying to understand Korean ciliates diversity, we

provide redescrptions briefly (Foissner, 1992; Foissner et al., 1995; Aesch, 2001).

### MATERIALS AND METHODS

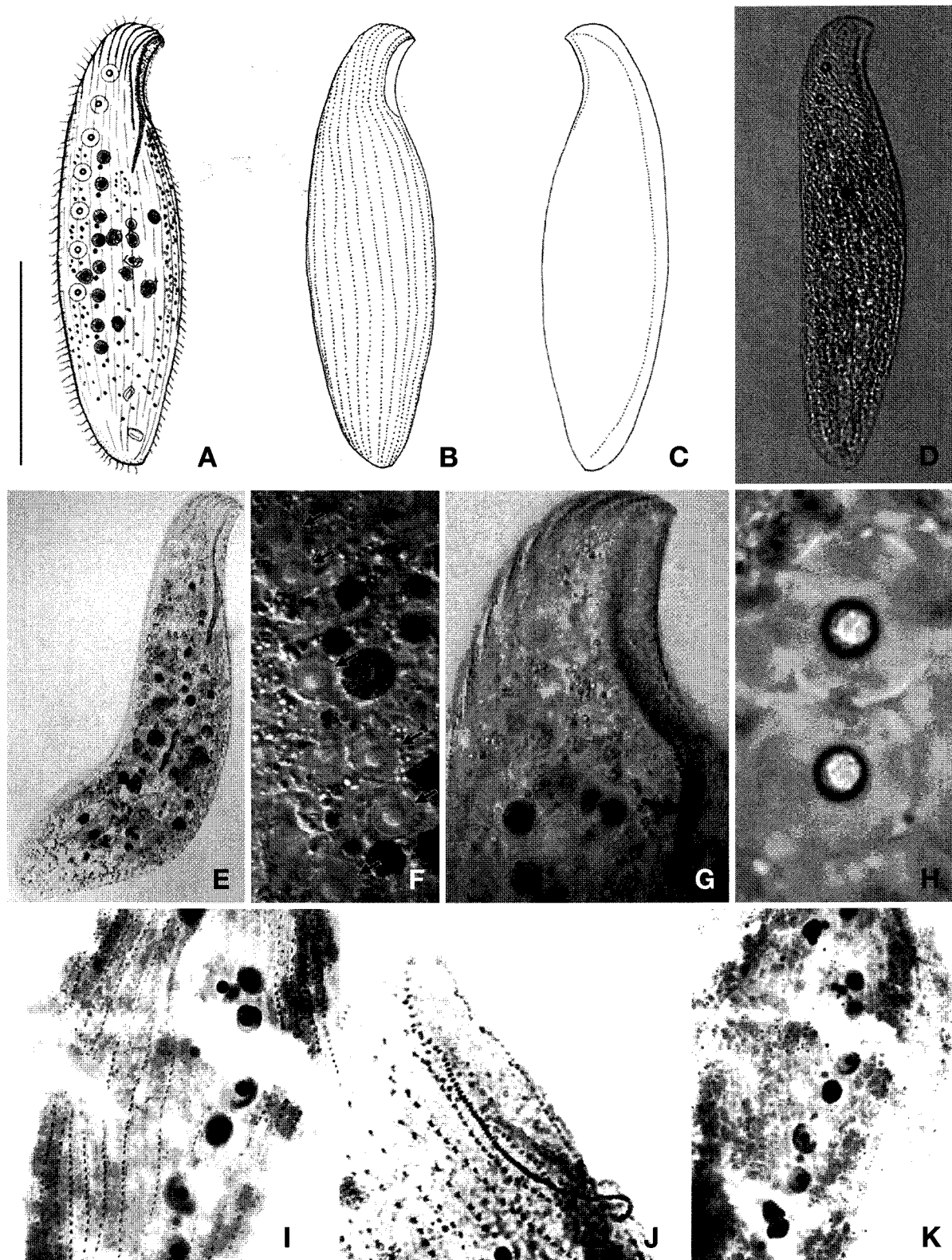
The collected specimens were moved to the laboratory and were isolated and cultured with dried wheat grains at room temperature. The living specimens were observed at low to high magnifications at bright field and differential interference contrast of optical microscope. The protargol silver staining method was applied with modifications to reveal the infraciliature and nuclear apparatus (Foissner, 1992). We adopted the classification schemes established by Lynn (2008).

### SYSTEMATIC ACCOUNTS

Phylum Ciliophora Doflein, 1901  
Subphylum Postciliodesmatophora Gerassimova and Servin, 1976  
Class Karyorelictea Corliss, 1974  
Order Loxodida Jankowski, 1980  
Family Loxodidae Büschli, 1889  
\*Genus *Loxodes* Ehrenberg, 1830  
Type species: *Loxodes rostrum* (Müller, 1773)

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**Fig. 1.** Morphology and infraciliature of *Loxodes kahli* Dragesco and Njiné, 1971 *in vivo* (A, D-H) and protargol impregnation (B, C, I-K). A, Right side view; B, Right side view of infraciliature; C, Left side view of infraciliature; D, Right side view; E, Food vacuoles; F, Macronuclei (arrows); G, Pharyngeal fiber (arrow); H, Müller's vesicles; I, Somatic kineties; J, Perioral kinety; K, Macronuclei and micronuclei. Scale bar=100  $\mu$ m.

**Table 1.** Morphometric data of *Loxodes kahli* (Lk), *L. magnus* (Lm) and *L. vorax* (Lv).

Characteristics	Species	Mean	Med.	Min.	Max.	SD	SE	CV	n
Body, length ( $\mu\text{m}$ ) <i>in vivo</i>	Lk	218	200	160	300	40.3	9.8	18.5	17
	Lm	350	339	250	470	74.7	17.6	21.3	18
	Lv	120	118	70	160	23.31	5.21	19.75	20
Body, width ( $\mu\text{m}$ ) <i>in vivo</i>	Lk	52	50	40	70	7.3	1.8	14.1	17
	Lm	111.2	107.5	87	159	24.7	5.8	22.2	18
	Lv	30	28.65	20	35	4.48	1.00	15.62	20
Body length/width, ratio	Lk	4.2	4.2	3.2	5.4	0.7	0.2	16.9	17
	Lm	3.2	3	2.3	4.1	0.58	0.14	18.06	18
	Lv	0.24	0.25	0.14	0.43	0.07	0.02	28.10	20
Müller vesicle, number <i>in vivo</i>	Lk	6	5	4	12	1.9	0.4	30.9	19
	Lm	13.5	14.5	8	18	3.1	0.7	23	18
	Lv	3	2.65	2	4	0.61	0.15	22.91	17
Mueller's vesicle, diameter ( $\mu\text{m}$ )	Lv	7	7.13	6.5	8	0.58	0.21	8.18	8
Cytostome, length ( $\mu\text{m}$ )	Lv	25	25.69	20	32	4.61	1.15	17.96	16
Cytostome length/Body length	Lv	0.22	0.23	0.14	0.38	0.07	0.02	30.61	16
Macronuclear nodule, length ( $\mu\text{m}$ )	Lk	5	5	4	7	0.7	0.2	14	19
Macronuclear nodule, width ( $\mu\text{m}$ )	Lk	5	5	4	6	0.5	0.1	9	19
Macronucleus, diameter ( $\mu\text{m}$ )	Lm	5.3	5.1	5	6	0.3	0.1	6.4	10
	Lv	6.0	6.4	5.0	7.5	0.73	0.20	11.39	13
Micronucleus, diameter ( $\mu\text{m}$ )	Lk	3	3	2	4	0.7	0.2	24.6	18
	Lm	3.5	3.5	3	4	0.4	0.1	12.7	10
	Lv	3	2.85	2	4	0.47	0.13	16.64	13
Macronuclei, number	Lk	8	8	6	11	1.3	0.3	15.8	18
	Lm	10.3	10	9	13	1.4	0.5	13.8	10
	Lv	2	2	2	2	0	0	0	20
Micronuclei, number	Lk	7	7	5	9	1.2	0.3	18	17
	Lm	10.1	10	8	13	1.4	0.5	14.4	10
	Lv	1	1	1	1	0	0	0	20
Pharyngeal fiber, length ( $\mu\text{m}$ )	Lk	59	60	50	70	8.9	4	15.2	5
Somatic kineties (right), number	Lk	19	19	18	20	0.8	0.2	4.2	12
	Lm	25	25	23	26	1.1	0.4	4.6	10
	Lv	6	5.83	5	7	0.72	0.21	12.30	12
Somatic kineties (left), number	Lm	2	2	2	2	0	0	0	10
	Lv	2	2	2	2	0	0	0	18

The abbreviations in the table are as follows: Min.=minimum; Max.=maximum; Med.=median; SD=standard deviation; SE=standard error; CV=coefficient of variation in %; n=population size.

#### <sup>1</sup>\*1. *Loxodes kahli* Dragesco & Njiné, 1971

(Fig. 1 and Tables 1, 2)

*Loxodes kahli* Dragesco & Njiné, 1971, p. 107; Dragesco & Dragesco-Kernéis, 1986, p. 207.

*Loxodes magnus* var. *penardi* Kahl, 1931, p. 215.

**Material examined.** These ciliates were collected from Tae-hwagang River in Ulsan (E 129° 16'27", N 35° 33'10") on 28th September 2007. 19 living and 18 protargol impregnated specimens were observed respectively and analyzed biometrically.

**Description.** General morphology. Cell *in vivo* 160-300 × 40-70  $\mu\text{m}$  (average 200 × 50  $\mu\text{m}$ ); length of cell about 4.2 times of width (Table 1). Body elongated with anterior portion forming slightly beak, posterior end conspicuous and slightly lanceolate (Fig. 1A, B, D). Oral aperture situated anteriorly, and narrow slit-like (Fig. 1A, D, E, G). Cytostome extending 26-33% of body length, contained reddish or dark-brownish pigments (Fig. 1G). Body color slightly brown at low magnification and sometimes green or yellow due to food vacuoles (Fig. 1E); food vacuoles scattered in entire body, and size about 10  $\mu\text{m}$  *in vivo*. Müller's vesicles arrang-

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**Table 2.** Comparison of diagnostic characters in related species of *Loxodes*.

Characters	Species							
	<i>L. kahli</i>	<i>L. kahli</i>	<i>L. magnus</i>	<i>L. magnus</i>	<i>L. rostrum</i>	<i>L. striatus</i>	<i>L. vorax</i>	<i>L. vorax</i>
Body length, <i>in vivo</i> (μm)	160-300	470-600	250-470	115-800 (400)	70-300	100-300 (200)	70-160	125-135
Body width, <i>in vivo</i> (μm)	40-70	—	87-159	40-200	—	—	20-35	—
Body color	Brown	Brown	Dark brown	—	—	Yellow	Yellowish brown	Yellowish brown
Somatic kineties, number (right)	18-20 (19)	13-26 (24)	23-26	25-32	10-12	9-13	5-7	—
Müller's vesicle, number	4-12	~10	8-18	10-24	2-6	4-12	2-4	—
Macronuclei, number	6-11	6-8	9-13	3-31 (11-23)	2	2	2	2
Micronuclei, number	5-9	6-8	8-13	9-32 (12)	1	2	1	1
Algal Symbionts	Absent	—	Absent	Absent	Present	—	Absent	Absent
Nuclear (Ma & Mi) arrangement	Macronuclei arranged linearly	Macronuclei arranged linearly	Macronuclei scattered	Macronuclei scattered	Micronucleus located between macronuclei	Micronuclei near macronuclei	Micronucleus located between macronuclei	Micronucleus located between macronuclei
Pharyngeal fiber	Directed dorsally	Directed dorsally	Directed dorsally	Directed dorsally	Directed dorsally	Directed dorsally	Directed posteriorly	Directed posteriorly
Reference	Present work	Dragesco and Njiné, 1971; Dragesco and Dragesco-Kernéis, 1986	Present work	Dragesco and Dragesco-Kernéis, 1986; Foissner et al., 1995	Dragesco and Dragesco-Kernéis, 1986	Dragesco and Dragesco-Kernéis, 1986	Present work	Kahl, 1931; Foissner et al., 1995

ed longitudinally from anterior half to middle half of dorsal side 4 to 12 (average 6) in number, and size about 7.5 μm and inner cores sized 2.5 μm *in vivo* (Fig. 1A, D, H). Contractile vacuole not detected. Somatic cilia about 7.5 μm in length.

**Infraciliature.** 18-20 somatic kineties on right surface and composed of dikinetids (Fig. 1A, B). Somatic kineties on left surface 2 in number, one located along the dorsal edge and other one located ventrally along oral region (Fig. 1B, C). Cilia mostly restricted to right (upper) surface and reduced on left (lower) surface, basal bodies of left surface monokinetid along dorsal and ventral edges.

**Nuclear apparatus.** Vesicular macronuclei positioned linearly and longitudinally; 6-11 (average 8) in number with 4-7 μm in diameter (Table 1 and Fig. 1A, I, K). Micronuclei 5-9 (average 7) in number; 2-4 μm in diameter; located near macronuclei (Table 1 and Fig. 1A).

**Microhabitat.** Sandy bottom in littoral of river, salinity of 0-0.01%.

**Distribution.** Africa, Europe and Korea.

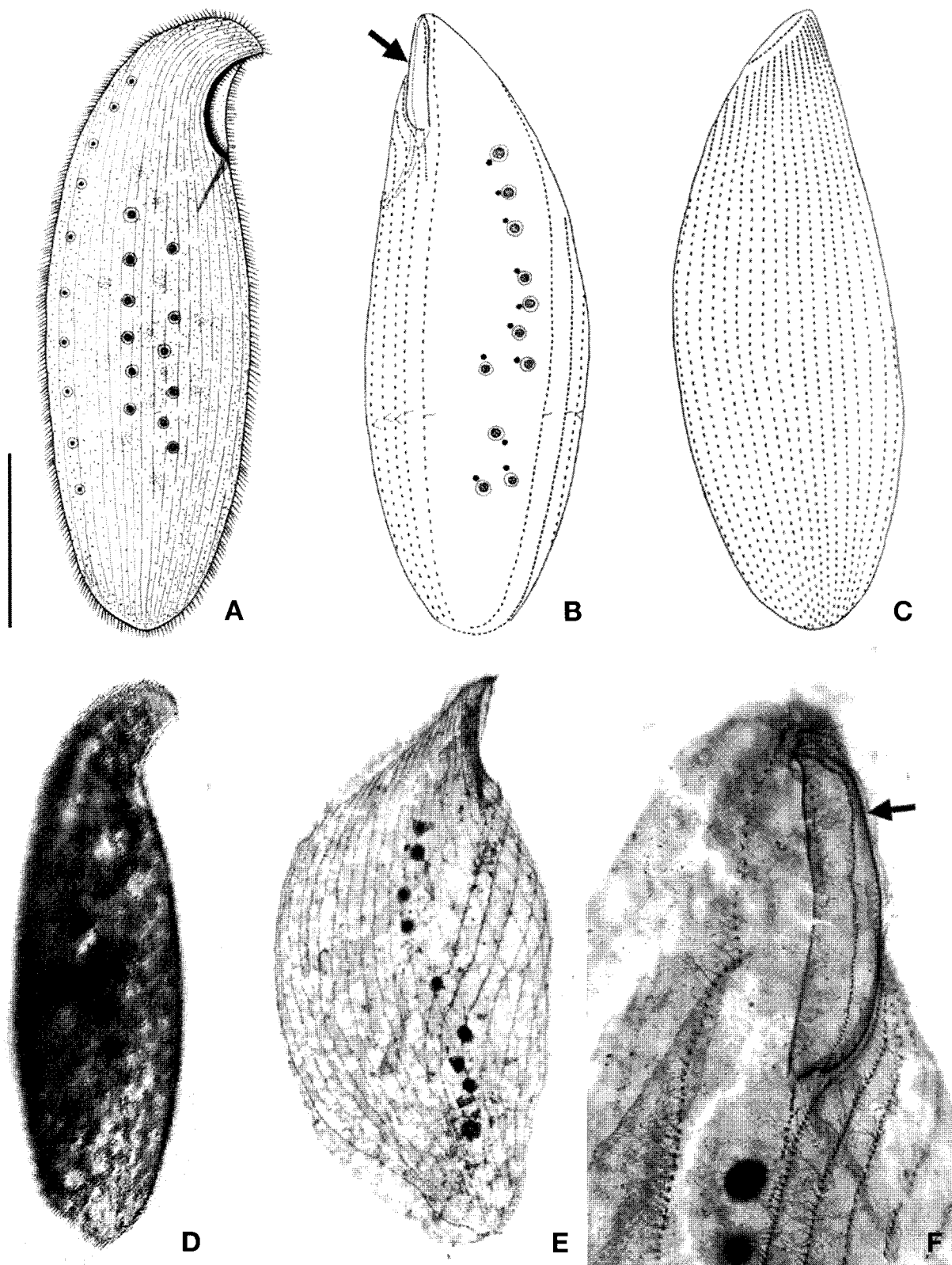
**Remarks.** The Korean population of *Loxodes kahli* matched well with the original and subsequent African populations with the exception of body size (160-300 vs. 470-600 μm). This species resembles *Loxodes magnus* Stokes, 1887 in terms of the body shape and body size. However, this species dif-

fers from the latter in several characteristics as follows (Table 2): 1) *L. kahli* has 6-11 macronuclei and 5-9 micronuclei, but *L. magnus* has 3-31 macronuclei and 9-21 micronuclei in African population, and 3-31 macronuclei and 2-32 micronuclei in European population. 2) *L. kahli* has 4-12 Müller's vesicles, but African and European populations of *L. magnus* 10-24. 3) *L. kahli* with 13-26 somatic kineties on the right surface, but *L. magnus* with 27-32 in African population and 25-32 in European population (Dragesco and Njiné, 1971; Dragesco and Dragesco-Kernéis, 1986; Foissner et al., 1995). Considering the number and arrangement of macronuclei (i.e. 6-13 in number and linear and longitudinal arrangement) and posteriorward direction of pharyngeal fiber, the variety of this species established by Kahl (1931), namely *L. magnus* var. *penardi*, might reasonable be regarded as the same species of this species.

<sup>1</sup>\*2. *Loxodes magnus* Stokes, 1887 (Fig. 2 and Tables 1, 2)  
*Loxodes magnus* Stokes, 1887, p. 106; Kahl, 1931, p. 215; 1935, p. 214; Dragesco, 1970, p. 15; Dragesco & Dragesco-Kernéis, 1986, p. 205; Carey, 1992, p. 91; Foissner et al., 1995, p. 378.

**Material examined.** These ciliates were collected from the

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**Fig. 2.** Morphology and infraciliature of *Loxodes magnus* Stokes, 1887 *in vivo* (A, D) and protargol impregnation (B, C, E, F). A, Right side view; B, Left side view; C, E, Right view of infraciliature; D, Swimming cell; F, Oral structure. Arrows (B, F) mark oral infraciliatures. Scale bar=100  $\mu$ m.

small pond in Changwon (E 128° 40' 10'', N 35° 17' 40'') on 12th February 2004. 18 living and 10 protargol impregnated specimens were observed respectively and analyzed biometrically.

**Description.** General morphology: Cell *in vivo* 250-470 × 87-159 µm (average 350 × 111.2 µm). Length of cell about 3.2 times of width (Table 1). Body elongated ellipsoidal to lanceolate (Fig. 2A, D). Body color dark brown. Anterior portion pointed and strongly bent ventrally forming concavity in which slit-like oral aperture situated (Fig. 2A, D). Posterior end of body rounded. Body compressed laterally in anterior portion but less in posterior portion (Fig. 2A). Contractile vacuole not detected. Müller's vesicles arranged longitudinally from anterior half to middle half of dorsal side 8-18 (average 13) in number (Fig. 2A). Oral aperture occupying about one-seventh of body length (Fig. 2A, D).

**Infraciliature:** 23-26 somatic kineties on right surface composed of dikinetids. Somatic kineties on left (lower) surface 2 in number, arranged along dorsal and ventral edges (Fig. 2B, C, E).

**Nuclear apparatus:** Macronuclei in vesicular parts 9-13 in number, 5-6 µm in diameter, arranged longitudinally in 2 rows in middle of body (Fig. 2A, B, E). Micronuclei 8-13 in number, 3-4 µm in diameter, located near macronuclei (Fig. 2B).

**Distribution.** Africa, Austria, England, Germany, United States, Japan and Korea.

**Remarks.** The characteristics of the Korean population of this species matched well with those of the original and other populations (Dragesco and Dragesco-Kernéis, 1986; Foissner et al., 1995). This species is similar to *Loxodes rostrum* (Müller, 1773) Ehrenberg, 1830 in terms of several characteristics, such as body shape, no contractile vacuole, and the location of oral aperture. However, *L. magnus* differs from *L. rostrum* in the following aspects: 1) The former has 9-13 macronuclei while the latter 2. 2) The former has 8-13 micronuclei while the latter 1. 3) The former has no symbiotic algae within the body while the latter has symbionts. 4) the former's body length is 250-470 µm while the latter 70-300 µm (Table 2) (Kahl, 1931; Dragesco and Dragesco-Kernéis, 1986; Foissner et al., 1995).

**<sup>1</sup>\*3. *Loxodes vorax* Stokes, 1885 (Fig. 3 and Tables 1, 2)**

*Loxodes vorax* Stokes, 1885 (cited from Kahl, 1931); Kahl, 1931, p. 213; Kudo, 1954, p. 727; Foissner et al., 1995, p. 393.

**Material examined.** These ciliates were collected from the wetland in Daegu (E 128° 29' 09'', N 35° 49' 17'') on 31st Octo-

ber 2008. 20 living and 18 protargol impregnated specimens were observed respectively and analyzed biometrically.

**Description.** General morphology: Cell *in vivo* 70-160 × 20-35 µm (average 120 × 30 µm). Length of body about 4 times of width (Table 1). Body elongated with anterior portion forming slightly beak, the posterior end conspicuous and slightly lanceolate (Fig. 3A, B, F). Body laterally compressed and slightly flexible (Fig. 3C). Oral aperture situated anteriorly and cytostome occupied 14-38% of body length, containing brownish pigments (Fig. 3A, F, G). Body color transparent but yellowish brown (Fig. 3F). Food vacuoles scattered in middle of body; anterior and posterior parts of cytoplasm without food vacuoles (Fig. 3F). Müller's vesicles 2-4 (average 3) in number, about 7 µm in diameter, and cores about 2.5 µm, and located in dorsal side of body (Fig. 3A, K). Cortical granules about 6 rows on each space between somatic kineties on right side; irregularly arranged on left side and size about 0.2-0.3 µm (Table 1 and Fig. 3I, J). Single contractile vacuole located near posterior part (Fig. 3A); size about 5-7.5 µm; contracting 1-2 times per 2 minutes. Cells usually crawling fast with straight body on sandy bottom but sometimes having twisted body when floating within water. Oral structures situated anteriorly and narrow slit-like shaped on ventral view (Fig. 3A, B, F, G). Cytostome extending 14-38% of body length contained yellowish brown pigments (Fig. 3A, F, G). Pharyngeal fiber directed posteriorly and arranged parallel with ventral edge (Fig. 3E). Oral cilia arranged along oral edge (Fig. 3A, F, M).

**Infraciliature:** 5-7 somatic kineties on right surface composed of dikinetids (Table 1). 2 somatic kineties on left surface composed of monokinetids, arranged along ventral and dorsal edges (Table 1, Fig. 3B, L).

**Nuclear apparatus:** 2 vesicular macronuclei situated in mid body with 5-7.5 µm in diameter (Table 1 and Fig. 3A, B, G, M). One micronucleus situated between macronuclei with 2-4 µm in diameter (Table 1 and Fig. 3A, B, G, M).

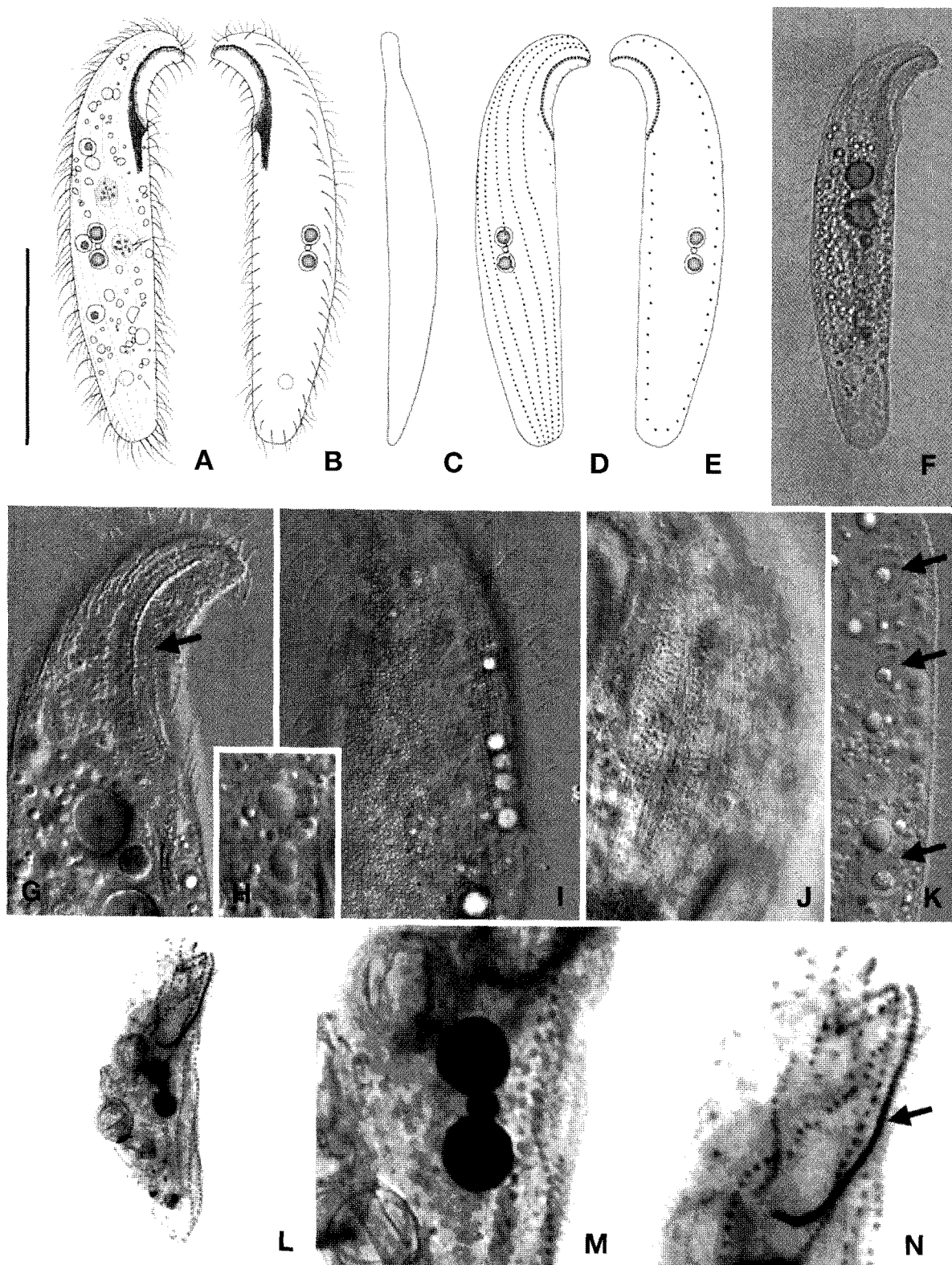
**Microhabitat.** Sandy bottom in wetland, freshwater.

**Distribution.** Africa, Europe, United States and Korea.

**Remarks.** The Korean and European populations of this species resemble each other in terms of several characteristics, such as body shape, direction of pharyngeal fiber, number and arrangement of nuclear apparatus and absence of symbionts (Kahl, 1931). The Korean specimens are very similar to *L. rostrum* (Müller, 1773) Ehrenberg, 1830. They have two macronuclei with 1 micronucleus between them (Table 2). However, *Loxodes vorax* differs from *L. rostrum* in the following aspects: 1) The former has 5-7 somatic kineties on the right side but the latter 10-12. 2) The direction of pharyngeal fiber in the former is dorsalward but posteriorward in

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**Fig. 3.** Morphology and infraciliature of *Loxodes vorax* Stokes, 1885 *in vivo* (A-C, F-K) and protargol impregnation (D, E, L-N). A, Right side view; B, Left side view; C, Dorsal view; D, Right side view; E, Left side view; F, Right side view; G, Oral structure (arrow); H, Macronuclei; I, Cortical granules on left side; J, Cortical granules on right side; K, Müller's vesicles (arrows) of dorsal side; L, Right side view; M, Macronuclei and micronucleus; N, Oral infraciliature (arrow). Scale bar=50  $\mu$ m.

the latter. 3) The former has symbiotic algae within the body but the latter has no symbiont (Table 2) (Kahl, 1931; Foissner et al., 1995).

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